PATENT
674523-2006.1

AMENDMENT

Please amend the application without prejudice, without admission, without surrender of subject matter, and without any intention of creating any estoppel as to equivalents, as follows.

IN THE SPECIFICATION

Additions

On page 1, line 1, please insert:

--TITLE OF THE INVENTION--

On page 1, line 2, please insert:

--CROSS REFERENCE TO RELATED APPLICATIONS

This is a divisional application of U.S. application Serial No. 09/238,356, filed on January 27, 1999, now U.S. Patent No. 6,312,683, issued on November 6, 2001, which is a continuation application of International application No. PCT/GB98/03876, filed on December 22, 1998, published as WO 99/32646 on July 1, 1999, and claiming priority to United Kingdom Application Nos. 9727135.7, filed on December 22, 1997 and 9811037.2, filed on May 22, 1998.

FIELD OF THE INVENTION--

On page 1, line 6, please insert:

--BACKGROUND OF THE INVENTION--

On page 2, line 19, please insert:

--SUMMARY OF THE INVENTION--;

On page 5, line 12, please insert:

--BRIEF DESCRIPTION OF DRAWINGS

Figure 1 shows the structure of transcription units from plasmids pESP, pONY3 and pONY2.1nlsLacZ. The genomic organization of EIAV is indicated including splice donor (d1, d2 and d3) and splice acceptor sites (a1, a2 and a3). The positions of *gag, pol, env, tat, rev, S2* and the viral LTRs are also shown. Plasmid pESP is an EIAV vector genome containing the SV40 promoter and the puromycin resistance gene. Plasmid pONY3 is an EIAV gagpol expression plasmid. pONY21nlslacZ is an EIAV vector genome containing a HCMV IE enhancer/promoter and a β-galactosidase gene (nlslacZ).

Figure 2 shows a PCR analysis of integrated EIAV vector. PCR was performed with

either genomic DNA from EIAV vector transduced cells (lanes 1 and 5) or mock transduced cells (lanes 2 and 6). pONY21nlsLacZ (lanes 3 and 7) and pONY3 (lanes 4 and 8) were used as controls. A) PCR detection of EIAV LTR. B) PCR detection of *pol*.

Figure 3 shows the structure of vector transcription units in deletion plasmids used to identify the packaging requirements for an EIAV vector.

Figure 4 shows a secondary structure prediction for the RNA derived from the *gag*-transcription unit (SEQ ID NO: 1) present in pONY2.13LacZ.

Figure 5 is a representation of vectors derived from the EIAV genome.

Figure 6 is a representation of gagpol constructs derived from EIAV.

Figure 7 is a representation of an EIAV vector comprising an *S2* deletion.

Figure 8 is a representation of EIAV *gagpol* constructs having deleted *S2* and *dUTPase* genes.

Figure 9 is a representation of an EIAV minimal vector.

Figure 10 shows analysis of gagpol expression constructs. 30μg of total cellular protein was separated by SDS/PAGE, transferred to nitrocellulose and probed with anti-EIAV antibodies. The secondary antibody was anti-Horse HRP (Sigma). Titers were averaged from three independent experiments and calculated as lacZ forming units per ml. There was no more than 10% variation between experiments. pONY2.1nlslacZ and the envelope expression plasmids were co-transfected with the EIAV *gagpol*.

Figure 11 shows examples of the pONY4 vectors.

Figure 12 shows two SIN vectors.

Figure 13 is a representation of a vector with a split polyA signal.

Figure 14 is a representation of a vector with a split polyA signal.

Figure 15 is a representation of a vector with a split polyA signal. A. SEQ ID NOs: 2 and 3. B SEQ ID NO: 4. C. SEQ ID NO: 5.

Figure 16 shows construction of pONY4-GFP with a split polyA signal.

Figure 17 shows construction of a MLV/EIAV vector.

Figure 18 shows primers for construction of MLV/EIAV vectors (SEQ ID NOs: 6-13).

Figures 19A and 19B show the complete sequence of pONYmouse (SEQ ID NO: 14).

Figures 20 and 21 show sequences of PCR primers EMVA 1-8 (SEQ ID NOs: 15-22).

Figure 22 shows pEMVA4 (after PCR with primers EMVA 1-8).

Figure 23 shows pEMVA4.

Figure 24 shows pEMVA5.

Figures 25 and 26 show an example of hammer-head strategy for 5' end formation (SEQ ID NOs: 23 and 24).

Figure 27 shows pEMVA6.

Figure 28 shows pEMVA7 and pSynPONY4.1.

Figure 29 shows EMVA 10/11 (SEQ ID NOs: 25 and 26).

Figure 30 shows pEMVA9.

Figure 31 shows pEMVA10.

Figure 32 shows pLWHORSE3.1.

Figure 33 shows pMCRev.

Figure 34 shows pYFVSVG.

Figure 35 shows pYFAmpho.

Figure 36 shows recombinant MVA constructs.

Figures 37A-37C show the complete sequence of pSC65 (SEQ ID NO: 27).

Figures 38A-38C show the complete sequence of pLW22 (SEQ ID NO: 28).

DETAILED DESCRIPTION OF THE INVENTION--;

Amendments

Please amend the paragraph on page 26, line 30, as follows:

--GCATGGACCTGTGGGGTTTTTATGAGG (SEQ ID NO: 29)--

Please amend the paragraphs beginning on page 27, line 1, as follows:

--GCATGAGCTCTGTAGGATCTCGAACAGAC (SEQ ID NO: 30)

The amplicon was blunt ended by 5' overhang fill-in inserted into pBluescript II KS+ cut with *BssHII* which had been blunt ended by 3' ~~overhand~~ overhang removal using T4 DNA polymerase. This construct was called pONY1 and the orientation was 5' to 3' in relation to β-galactosidase of pBluescript KS+. Sequencing of pONY1 revealed no mutations.--

Please amend the paragraph beginning on page 34, line 7, as follows:

--Sequences encompassing the EIAV polypurine tract (PPT) and the 3' LTR were obtained by PCR amplification from pONY2.10LacZ using primers PPTEIAV+ (Y8198):

GACTACGACTAGTGTATGTTTAGAAAAACAAGG (SEQ ID NO: 31), and 3'NEGSpeI (Y8199): CTAGGCTACTAGTACTGTAGGATCTCGAACAG (SEQ ID NO: 32). The product was purified, digested with *SpeI* (ACTAGT) and ligated into pBS II KS+ which had been prepared by digestion with *SpeI* and treatment with alkaline phosphatase. Colonies obtained following transformation into E. coli, XL-1 Blue were screened for the presence of the 3' LTR in the orientation in which the U5 region of the 3' LTR was proximal to the *NotI* site of the pBS II KS+ linker. The sequence of the cloned insert was determined and showed that it contained only one change from the EIAV clone pSPEIAV19 (AC:U01866). This was a 'C' insertion between bases 3 and 4 of the R region. The same change was found in the template used in the PCR reaction. The clone was termed pBS.3'LTR.--

Please amend the paragraphs beginning on page 35, line 1, as follows:

--The 5' region of the EIAV vector was constructed in the expression vector pCIEneo which is a derivative of pCIneo (Promega) modified by the inclusion of approximately 400 base pairs derived from the 5' end of the full CMV promoter as defined previously. This 400 base pair fragment was obtained by PCR amplification using primers VSAT1 (GGGCTATATGAGATCTTGAATAATAAAATGTGT (SEQ ID NO: 33)) and VSAT2 (TATTAATAACTAGT (SEQ ID NO: 34)) and pHIT60 as template. The product was digested was *BglII* and *SpeI* and ligated into pCIneo which had been digested similarly.

A fragment of the EIAV genome running from the R region to nt 150 of the *gag* coding region (nt 268 to 675) was amplified with primers CMV5'EIAV2 (Z0591) (GCTACGCAGAGCTCGTTTAGTGAACCGGGCACTCAGATTCTG (SEQ ID NO: 35))[[:] and 3'PSI.NEG (GCTGAGCTCTAGAGTCCTTTTCTTTTACAAAGTTGG (SEQ ID NO: 36)) using template DNA. The 5' region of the primer CMV5'EIAV2 contains the sequences immediately upstream of the CMV promoter transcriptional start site and can be cut with *SacI*. 3'PSI.NEG binds 3' of the EIAV packaging sequences as defined by deletion analysis (above) and contains an *XbaI* site. The PCR product was trimmed with *SacI* and *XbaI* and ligated into pCIEneo which had been prepared for ligation by digestion with the same enzymes. This manipulation places the start of the EIAV R region at the transcriptional start point of the CMV promoter and transcripts produced thus start at the genuine start position used by EIAV and extend to the 3'-side of the packaging signal. Clones which appeared to be correct as assessed by restriction analysis were sequenced. A clone termed pCIEneo.5'EIAV was selected for further

work.--

Please amend the paragraphs beginning on page 36, line 17, as follows:

--The HIV-1 RRE was obtained from the HIV-1 molecular clone pWI3 (Kimpton and Emerman 1992 (J. Virol. 66: 2232-2239) by PCR amplification using primers RRE(+) GTCGCTGAGGTCGACAAGGCAAAGAGAAGAG (SEQ ID NO: 37) and RRE(-) GACCGGTACCGTCGACAAGGCACAGCAGTGG (SEQ ID NO: 38). The fragment of DNA and pEGASUS-1 were digested with *SalI* and following ligation, transformed into E. coli, XL-1 Blue. Colonies were screened for the presence of the HIV RRE and two clones, with the HIV RRE in either the positive or negative orientation, used for further work. These vectors, pEGASUS-2.HIV RRE(+) or pEGASUS-2.HIV RRE(-) can be tested in 293T cells by carrying out a four plasmid co-transfection in which the plasmid pCIneoHIVrev, expressing the rev protein from HIV-1 is co-transfected with vector, pONY3 and pRV67 plasmids.

The EIAV RRE as defined previously (Martarano et al 1994) was obtained by PCR amplification as follows. Using pONY2.10LacZ as template 2 amplifications were performed to obtain the two parts of the EIAV RRE. The 5'-element was obtained using primers ERRE1 (TTCTGTCGACGAATCCCAGGGGGAATCTCAA) (SEQ ID NO: 39)) and EREE2 (GTCACCTTCCAGAGGGCCCTGGCTAAGCATAACAG (SEQ ID NO: 40)) and the 3' element with ERRE3 (CTGTTATGCTTAGCCAGGGCCCTCTGGAAGGTGAC (SEQ ID NO: 41)) and ERRE4 (AATTGCTGACCCCCAAAATAGCCATAAG (SEQ ID NO: 42)). These products will anneal to each other, and hence can be used in a second PCR reaction to obtain a DNA which 'encodes' the EIAV RRE. The PCR amplification is set up ~~without~~ without primers ERRE1 and ERRE4 for the first 10 cycles and then these primers are added to the reaction and a further 10 cycles of amplification are carried out. The resulting PCR product and pEGASUS-1 were digested with *SalI*, ligated and transformed into E. coli XL-1Blue. Clones in which the EIAV RRE was in either the positive or negative orientations were selected for further work. The activity of these vectors was assessed in three ~~palsmid~~ plasmid co-transfection experiments as described above, but using pCIneo.EIAV Rev to supply additional EIAV rev.

For construction of pCIneo EIAV REV, the EIAV REV encoding sequences were derived by PCR amplification. The EIAV REV sequences were obtained using a two step 'overlapping' PCR amplification procedure, as described above for the EIAV RRE. Template for the two reactions was pONY3 and primers for the 5' fragment were EIAV REV5'O

(CCATGCACGTCTGCAGCCAGCATGGCAGAATCGAAG (SEQ ID NO: 43)) and EIAV REV IN (CCTGAGGATCTATTTTCCACCAGTCATTTC (SEQ ID NO: 44)) and for the 3' product EIAV REV IP (GTGGAAAATAGATCCTCAGGGCCCTCTGG (SEQ ID NO: 45)) and EIAV.REV3'O (GCAGTGCCGGATCCTCATAAATGTTTCCTCCTTCG (SEQ ID NO: 46)). The second PCR amplification was carried out, with primers EIAV REV5'O and EIAV REV3'O being added after 10 cycles. The resulting product was ligated with the PCR fragment 'TA' cloning vector pCR2.1 (Invitrogen). The the orientation of the EIAV REV insert was assessed by restriction enzyme analysis and the presence of the correct EIAV REV sequence was confirmed. The construct was called pTopoRevpos. The EIAV REV insert was excised from pTopoRevpos by digestion with *SpeI* and *NotI* and ligated into pCIneo, which had been digested with *NheI* and *NotI*.--

Please amend the paragraph beginning on page 48, line 1, as follows:

--Transfections were carried out in 293T cells with pCI-Rev and pRV67. The virus was titred on D17 cells.--

Please amend the paragraph on page 49, line 11, as follows:

--**SY1:** GAC A CCATGG GAA GTA TTT ATC AC (SEQ ID NO: 47) (*Nco*I underlined)--

Please amend the paragraph on page 49, line 14, as follows:

--**SY2:** CCT GGG ATT CAT ATC AAA CCT TAT AAC AAA TAT TG (SEQ ID NO: 48)--

Please amend the paragraph on page 49, line 19, as follows:

--**SY3:** TCC T GCTAAGC ATA ACA GAA AC (SEQ ID NO: 49) (*Cel* II underlined)--

Please amend the paragraph on page 49, line 22, as follows:

--**SY4:** GGT TTG ATAT GAA TCC CAG GGG GAA TCTC (SEQ ID NO: 50)--

Please amend the paragraph on page 49, line 25, as follows:

--**SY5:** ACCC CGTACG TCT TCC CGA GCG (SEQ ID NO: 51) (*Sun* I underlined)--

Please amend the paragraph on page 49, line 26, as follows:

--dUTPaseF: GTTATTAATTAATGGAGGAATAATTGAAGAAGGATATAC (SEQ ID NO: 52) (*Pac* I underlined)--

Please amend the paragraphs beginning on page 50, line 5, as follows:

--dUTPaseR: TCTTCTGCAGGTCCTGATCCTTGCTTAGTGC (SEQ ID NO: 53)
(*Pst* I underlined)

S2StopR: GACCATGTTACCCCTTTACCATTAACTCCCTAATATCAAAC (SEQ ID NO: 54)
(The bases in bold are the base changes from TATGG to TTAGG that remove the start codon of S2).

S2StopF: GTAAAGGGGTAACATGGTCAGCATCGCATTCTACGGGGGAATCC (SEQ ID NO: 55)
(The base in bold is the base change from TATGG to TACGG that remove the start codon of S2).

EGAGP5'OUTER: CCATGCACGTCTCGAGCCAGCATGGGAGACCCTTTGAC (SEQ ID NO: 56)
(*Xho* I underlined)

EGAGP3'OUTER:
CGAGCTAGAGGTCGACTCAATTTGGTTTATTAGTAAC (SEQ ID NO: 57)
(*Sal* I underlined)

EGAGPINNER3: GCAATGGAATGACATCCCTCAGCTGCCAGTCC (SEQ ID NO: 58)
(*Pvu* II underlined)

EGAGPINNER5:
GGGATGTCATTCCATTGCCACCATGGGAAGTATTTATCACTA (SEQ ID NO: 59)
(*Nco* I underlined)--

Please amend the paragraphs beginning on page 52, line 27, as follows:

--DNA sequences between nucleotides 7300 and 8079 (numbered according to EIAV clone pSPEAIV19, Accession No. U01866) were obtained using polymerase chain reaction

amplification using pONY4G as template. The positive sense primer was ERRE3 and the negative primers for amplification were

SIN-MLU (C7143: GTCGAGC**ACGCGT**TTGCCTAGCAACATGAGCTAG (SEQ ID NO: 60) (*MluI* site in bold) or

SIN-MUN (C7142: GTCGAGC**CAATTG**TTGCCTAGCAACATGAGCTAG (SEQ ID NO: 61) (*MunI* site in bold) where the underlined sequences are complimentary to nucleotides 8058 to 8079 (of pSPEIAV19). The PCR products were digested with *NspV* and either *MluI* or *MunI* respectively. These were then ligated into pONY4G prepared for ligation by digestion with *NspV* (*SfuI*) and either *MluI* (partial digestion) or *MunI* respectively.--

Please amend the paragraph beginning on page 57, line 25, as follows:

--Figure 11 is a schematic representation of the EIAV genome vectors pONY4.0 and pONY4.1 which have been described in Example 10 and the vaccinia transfer vector pSC65 (Chakrabarti et al 1997). The P7.5E sequence is AAAAGTAGAAAATATATTCTAATTTATT (SEQ ID NO: 62). The Early termination sequence for the early promoters is TTTTTNT (N= any nucleotide) (Fields).--

Please amend the paragraph beginning on page 60, line 11, as follows:

--The T7 promoter has the sequence (-)TAATACGACTCACTATAGG(+2) (SEQ ID NO: 63) with transcription beginning after A with preferably a run of Gs. The T7 termination sequence is CTAGCATAACCCCTTGGGGCCTCTAAACGGGTCTTGAGGGGTTTTTTG (SEQ ID NO: 64). The T7 promoter and terminator sequences are as those described in the plasmid pCITE-4a(+) (Novagen).--

Deletions

Please delete the section of the specification from page 23, line 1, to page 25, line 14, without prejudice.